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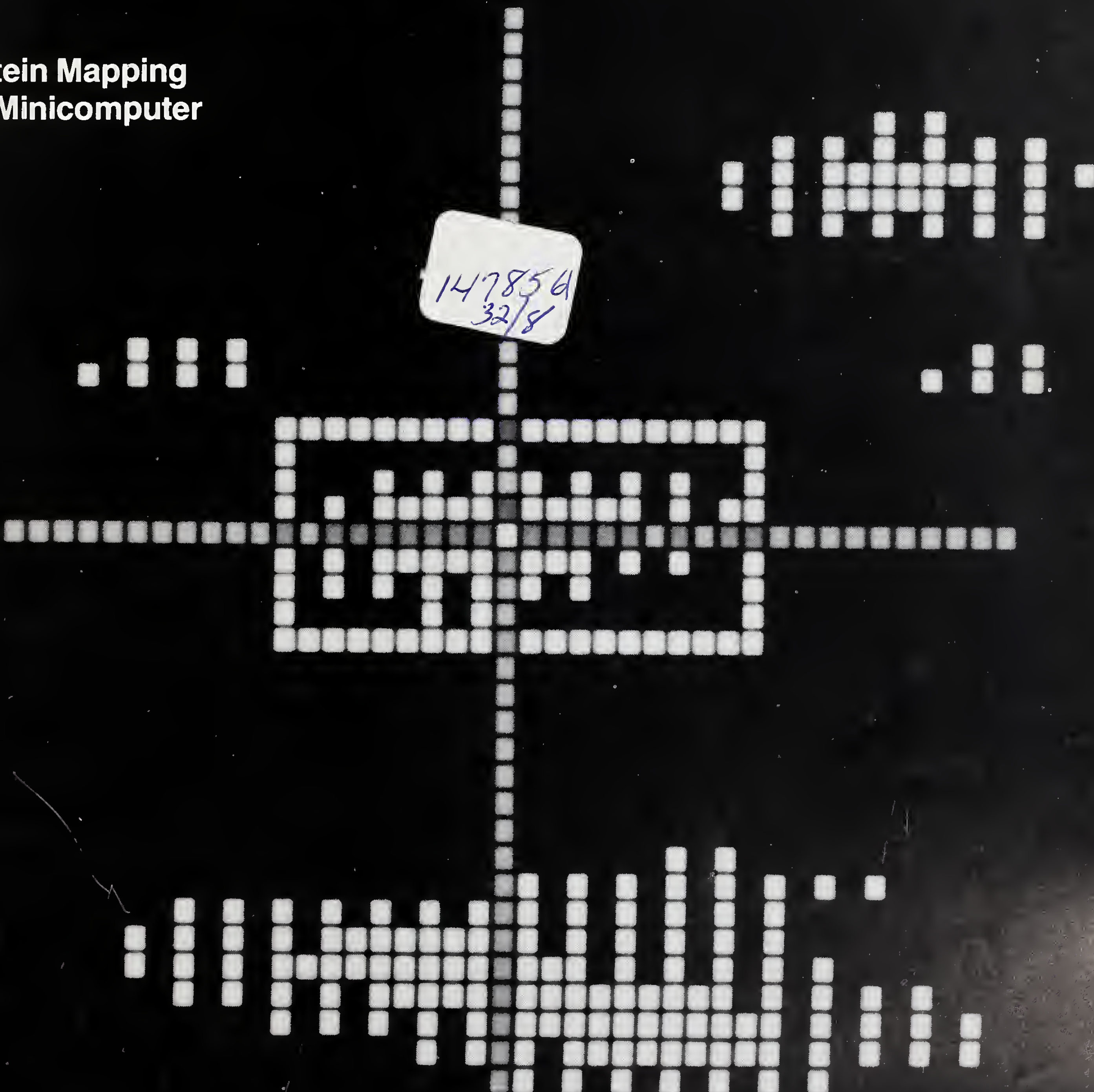
Agricultural Research

Protein Mapping Via Minicomputer



A Song-Filled Spring

A tremor traversed buildings along Independence Avenue in the Nation's Capital that house the U.S. Department of Agriculture, shaking up occupants in June of 1962.

It wasn't an earthquake. It was reaction to a series of articles in the *New Yorker* by Rachel Carson from her book, *Silent Spring*, that shook research officials.

Like a good country lawyer, the well-known author molded a case *against* agricultural chemicals because of their effect on the environment and *for* using nature's ways of controlling plant pests.

Public reaction was immediate, loud, and prolonged.

"The effects are still being felt, more than two decades later, and most have been positive," says Terry B. Kinney, ARS administrator. "Through citizen support, and influence on Congress, the late Ms. Carson literally turned around the research budgeting scene for a better research balance. However, it is important to remember that the Department had been doing research on biological pest control since 1888, when introduction of the vedalia beetle saved the California citrus industry from widespread losses to a scale insect."

Since *Silent Spring* was published, ARS has increasingly devoted more of its research effort away from chemical approaches and toward alternate methods of pest control. In ARS's proposed 1985 budget, nearly 80 percent of funding for pest control research is aimed at finding biological and other nonchemical controls.

What some people construed then as an attack on the research system was in reality a positive and constructive force for basic and applied research into environmentally clean control methods. As a result of this public support, the agricultural research community, both public and private, has made giant strides in devising alternate controls to chemicals. But these strides take years.

Two research accomplishments, each requiring 15 to 20 years from concept to introduction, illustrate this point.

- Farmers now have available to them, for the first time in history, a viral insecticide—*Heliothis* NPV (nuclear polyhedrosis virus)—to control two major *Heliothis* pests in cotton, corn, soybeans, sorghum, tomatoes, and tobacco. Together, these six commodities occupy 45 percent of U.S. cropland each year. (See *Agricultural Research*, Jan. 1984, p. 12.)
- The cereal leaf beetle—once a scourge of wheat and other small grain crops in the United States—was brought under control by the early 1980's, thanks largely to parasitic wasps introduced from Europe. This is the first instance in which a pest of an annual crop grown in a temperate continental area has been successfully controlled by imported natural enemies, ARS entomologists say. (See *Agricultural Research*, Nov. 1982, p. 8.)

"We have reason to hope that in the long term the savings in farm production costs and protection of the environment will exceed the cost of research and development of biocontrol agents many times," says Kinney. "Ms. Carson added the necessary impetus to our need for a more balanced program. That balance, however, will include the use of pesticides, which still have an important role to play, especially in finely tuned, integrated pest management programs."

For example, in the case of the cereal leaf beetle, pesticides are still necessary at the outer periphery of the beetle's spread in order to control the pest's advance until the predator wasps within the periphery become established.

In combination with research to find alternatives to pesticides is a research effort to target pesticides more precisely and in less quantity.

The natural, nontoxic pheromones that one sex of a species emits to attract a mate are already reducing the amount of pesticides needed to control insects. Science has identified and reproduced pheromones for hundreds of insect pests. They are being put to use to monitor insect populations so that farmers and foresters can apply pesticides only when they are needed and most likely to be effective. Pheromones are becoming increasingly important in pest control as entomologists learn more about the life cycles of various insect pests and chemists develop better ways to formulate pheromones for slow release.

Cotton pests can be lured to their death by combining smaller doses of insecticides with pheromones and feeding stimulants. (See *Agricultural Research*, July-Aug. 1982, p.4.) And an increasing number of insect pests are finding it difficult to locate a mate (and thus produce another generation) when the air about them is saturated with man-made pheromones.

Agricultural leaders need to convince critics of the many steps and years involved in basic research and development before nonchemical controls can be used safely and effectively as substitutes for chemicals.

It took about 15 years, from 1961 to 1975, from concept by an ARS insect pathologist to approval by the Environmental Protection Agency of the viral insecticide, *Heliothis* NPV, against the destructive budworms and bollworms that attack cotton. It took a similar period for the bacterial insecticide, *Bacillus thuringiensis (Bt)*, to be developed to the point of use.

Now scientists have discovered that *Bt* is effective against some of our most destructive insects, including the gypsy moth that has infested and defoliated many thousands of acres of U.S. woodlands.

Had the state of the art been where it is today when DDT was banned, we could have found a way to phase out DDT gradually and introduce *Bt* and other alternatives to continue control without missing a step, or perhaps even a springtime note.

Robert E. Enlow
Peoria, Ill.

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Cover: A minicomputer zooms in on one of hundreds of soybean seed proteins lodged in a "protein map." To allow more scientists to study plant and animal proteins via the sophisticated technique of protein mapping, ARS scientists at Beltsville, Md., developed a program for economical minicomputers instead of expensive mainframe computers now being used in this research. Story on page 4. (0484W485-7)

Taking the Byte Out of Protein Mapping

Plant physiologist James D. Anderson and biological lab technician Tommie L. Johnson identify soybean cultivars by examining differences in their protein profiles in the gels. (0484X402-29)

At first glance, William R. Hruschka's laboratory looks like an electronics repair shop with a computer graphics terminal tossed in for good measure. But that terminal is the focus of his research. He uses it to instruct a minicomputer to read and compare "maps" of plant proteins.

Hruschka turns on the system and the graphics terminal projects an array of irregular gray spots that represent soybean seed proteins. After he types in a command, the screen zeros in on a section of the map for more detail. With another command, a second screen comes to life and indicates the quantities of the proteins along a selected longitude or latitude. Still another command, and a third screen lights up to display the spots in a full range of reds or greens. The proteins in two maps can be compared by superimposing a green map over a red map. Wherever the proteins overlap they appear yellow.

Hruschka, an ARS mathematician with Beltsville's Instrumentation Laboratory in Maryland, developed the program to enable more scientists to benefit from the sophisticated technique of protein mapping. And it is proving its usefulness in many other applications.

Protein mapping is an invaluable tool for understanding the chemistry of living organisms, explains Hruschka, but it would be a superhuman task to track the comings and goings of the thousands of proteins produced by a single plant—and to read the quantity of each protein—without a computer. In the past, the task of tracking proteins has been assigned exclusively to large mainframe computers that have many times more memory and are far more expensive.

Budgetwise, minicomputers are within reach of many laboratories that don't have access to a mainframe, says Hruschka, pointing out that his program could also be used with a mainframe. The large computers, however, have one advantage. Because of their storage capacity, they can compare 10 or more maps at once, whereas Hruschka's minicomputer can

While Anderson watches, William R. Hruschka commands his minicomputer to activate the two screens on the right. The top screen shows the percent distribution of soybean seed proteins along one of the map's coordinates, while the bottom screen projects two maps simultaneously. By rapidly alternating the two maps on the screen with a "flicker" command, the scientists can spot which proteins differ between the two maps because they appear to move. (1083W1349-5)

compare only 2. With 400K bytes of memory, the minicomputer has three to six times the memory of the latest home computers.

A computerized system for tracking proteins "has enormous potential in agriculture alone," explains James D. Anderson, plant physiologist and chief of the Plant Hormone Laboratory at Beltsville. Plant scientists can get a much clearer picture of how, when, and where plant cells manufacture proteins, what the proteins do, and how environmental stresses, aging, or applied chemicals affect plant chemistry. Plant breeders can use computerized protein maps to fingerprint different varieties of plants because each has a slightly different protein profile. The maps could also be useful in recombinant DNA experiments to find the new proteins produced when a gene is spliced into the DNA of an organism. And food inspectors could use maps of meat proteins to check for adulteration in processed meats.

As far as they know, Hruschka, Anderson, and colleague David R. Massie, an electronics engineer with the Instrumentation Laboratory, are the only researchers who have developed a system for agricultural use. It is also the only system designed for minicomputers.

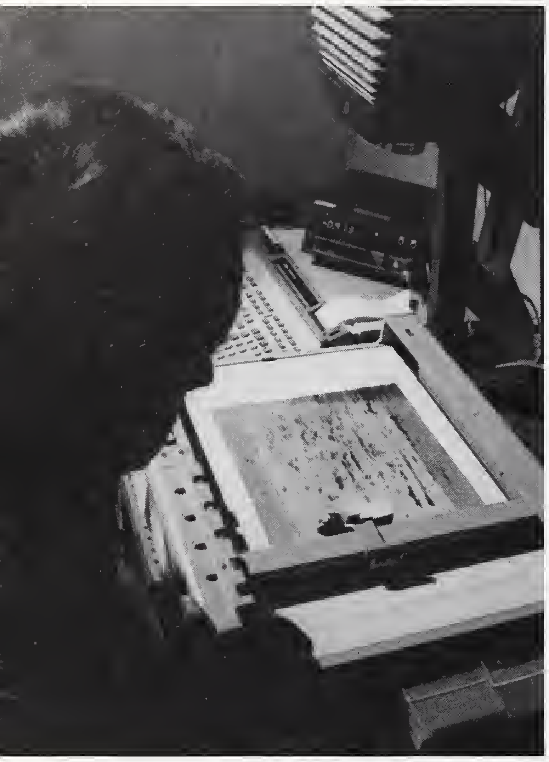
Hruschka says his program uses simpler math than other available programs and therefore works faster because "it gives the computer fewer instructions." It requires 400K bytes of memory to store the maps and operate the system, he says, but it could be altered (with some loss of speed) to use a magnetic disk for storing the maps if the researcher already has a computer with less memory. Moreover, researchers with little or no prior computer experience can use the program by making choices from a series of menus. As an added bonus, the program is free to interested scientists because Hruschka is a federal employee.

On the floor above Hruschka's lab, Anderson extracts proteins from plant tissue and prepares the maps using the technique developed in the mid-1970's by Patrick O'Farrell, now at the University of California, San Francisco. Called two-dimensional polyacrylamide gel electrophoresis, or 2-D PAGE for short, the technique is a two-stage process that separates proteins in one direction by their electrical charges, then at a 90° angle by their molecular weights. It might be compared to sorting a deck of cards in one direction by suits (electrical charge) and in a perpendicular direction by card value (molecular weight).

All proteins have numerous tiny electrical charges that add up to a net charge, but only a few proteins have the same net charge. When Anderson adds a plant extract to the top of a pencil-size column of gel and charges the column with an electrical current, the proteins move down at different speeds depending on their net charges and collect in discrete bands. Each band generally represents a different charge although there is some overlap.

Anderson then lays the gel column on its side across the top of a sheet of polyacrylamide gel less than a millimeter thick held between glass plates. Picture a spiral notebook (the gel sheet) standing on edge with its coil (the gel column) running horizontally across the top edge. The gel sheet contains a detergent that masks the net charges of individual proteins with its own negative charge. When an electrical current passes through the gel sheet, the proteins again move down, but this time according to their molecular weight since all now have the same net charge.

The smallest proteins move the quickest and end up near the "finish line." The largest proteins barely make it across the "starting line" and the middleweights separate accordingly somewhere in between. Thus the pro-



Electronics engineer David R. Massie sets up his "economy" digitizer to translate a gel of soybean seed proteins into computer language. (1083W1051-25A)

teins sort out horizontally by their net electrical charge and vertically by their molecular weight. Their positions on these coordinates identify them. They are mapped.

The proteins in the gel are made visible either by a radioactive process or with a stain, depending on what one is looking for. In the radioactive process, Anderson incubates living plant tissue in water containing a radioactive amino acid. The "hot" amino acid is absorbed by the tissue and becomes part of any protein the cell is constructing while the tissue is incubating, he explains. After he maps the proteins he exposes the dried gel to film, which records the radioactive proteins as dark spots. The exposed film, called an autoradiograph, looks like a miniature X-ray.

When he doen't use the radioactive process, Anderson stains the finished gel with a silver compound. Either way, the proteins appear as dark or shadowy spots.

Each method has its advantages. With "hot" amino acids, Anderson can "catch only the proteins the plant is synthesizing at a particular time and compare them with proteins the plant tissue is synthesizing at another time." These stop-action shots could eventually give scientists a kind of motion picture of the comings and goings of plant proteins.

By contrast, stains are not so selective. They bind to virtually all plant proteins and indicate which ones are present. Plant breeders can use these profiles to classify or identify cultivars.

Anderson prepares his own polyacrylamide gels, but even under his experienced hand the gels are not always uniform. Some areas within the gel sheet or between two sheets may be more porous than others, allowing proteins to slip further than normal. "It's as much an art as it is a science to make a good gel plate," he says.

Hruschka has instructed the computer to account for these small inconsistencies in position by adapting a technique developed by scientists at the Argonne National Laboratories in Illinois. Called rubber sheeting, the technique allows the computer to electronically stretch the gel images so that identical proteins in different gels line up one over the other. Hruschka says this has been his biggest challenge in programming the system.

The third member of the team, David Massie, acts as an intermediary between Anderson and Hruschka. Because computers deal only in numbers, not pictures, Hruschka's computer can't digest Anderson's gels. So Massie designed an inexpensive digitizer to translate the protein spots on Anderson's gels into number values.

In keeping with the team's economy-mindedness, Massie built the digitizer out of "equipment you would ordinarily find around a lab," Hruschka quips. Commercial digitizers, by contrast, cost between $20,000 and $30,000. Massie's digitizer combines an ordinary photographic enlarger, a silicon phototransistor, a programmable plotter, and a desktop calculator. Although it takes 7 hours to translate one gel compared with a few minutes for the commercial types, it is more accurate, says Massie, referring to its ability to accurately measure the quantity of individual proteins in the dark and shadowy spots on Anderson's gels.

It does so by measuring the intensity of light that passes through 65,536 imaginary points (pixels) on each gel, and translates each point into a number value on a tape. Once the tape is fed into the computer, Hruschka can call up the image on his graphics terminal, zoom in on a particular protein and ask the computer for measurement. The whole system from the two-stage protein separation through the digitizer to the computer can detect as little as one one-hundred-millionth (10^{-8}) of a gram of protein.

Without the digitizer, the program is proving quite useful in other applications, says Hruschka. When lower accuracy is acceptable, he and Massie use a TV camera to measure light density of gels, photographs, and negatives in one-thirtieth of a second. So far, they have quantified the amount of sunlight that penetrates a tree canopy and accurately measured the vitreous material in wheat that indicates its protein content as well as its milling quality.

"Eventually," Anderson muses, "I can see the computer scanning the gels, storing the information, and doing the comparisons automatically, but that day is way down the pike."

Maybe not. Look what happened with economy cars.

William Hruschka, James Anderson, and David Massie are located in Bldg. 002, Beltsville Agricultural Research Center-West, Beltsville, Md. 20705— **Judy McBride,** Beltsville, Md. ■

Zinc May Be Important During Pregnancy

Diets mildly deficient in zinc caused memory and learning impairments in the offspring of laboratory rats fed the diets during pregnancy and suckling periods. And the impaired learning continued into adulthood, says ARS research psychologist Edward S. Halas. He conducted the studies at the Grand Forks Human Nutrition Research Center in North Dakota.

The findings may have implications for humans, Halas says.

To diagnose the impairments, Halas used a maze patterned after one developed by researchers at Johns Hopkins University to study effects of surgically imposed injuries on the rat brain. The Johns Hopkins researchers found that injuries to the hippocampus area of the brain impaired short- and long-term memory.

In the Grand Forks study, Halas says, "We found that the hippocampus areas were less developed in zinc-deficient rats with memory and learning impairments than they were in the rats on normal diets."

In rats and in humans, the hippocampus normally has high concentrations of zinc, a trace mineral that is essential for formation of nucleic acids and protein. Whether zinc deficiency in human fetuses interferes with the rapidly developing hippocampus during pregnancy and postnatal periods is not known, says Halas. But he suggested that it may be prudent for pregnant women to consume foods rich in zinc. Good sources of the mineral include oysters, variety meats such as liver or beef heart, other kinds of beef, dark poultry meat, and crab.

Once the brain is fully developed in a rat or child it is difficult to injure it by nutritional means, Halas says. But if zinc deficiency occurs early in life during the critical period of brain development, he adds, normal growth and maturation may be irreversibly impaired.

Can zinc-deficient rats be rehabilitated in other ways?

"Insights for answering that question may depend on whether learning impairments in rats are general or specific in nature," says Halas. "If they're specific, perhaps, in other kinds of learning experiments, the rats could be rehabilitated to learn through kinds of stimuli that are different than the ones involved in our study. Knowledge is too limited to comment on humans."

The rat maze Halas used can test for either short-term memory or learning, depending on how it is used. With 17 radial arms, it resembles the hub and spokes of a wheel.

"If you're testing for memory you put a pellet of food at the end of every arm of the maze," Halas explains. "After a rat has gone to several arms he has to remember which arms he's already visited if he is to be successful in finding food on the next trip away from the hub. A failure to find food on a trip constitutes an error of short-term memory."

In the test for learning only 8 of the 17 arms are baited for a 15-minute period each day. The other nine arms are never baited so the rat has two problems: to remember which arms have been baited and which arms were visited recently. Successfully solving both problems constitutes learning.

The rats were always motivated for the experiments because the few pellets they got during the tests were only part of their daily ration—not enough to satisfy them. During the rest of the day they received sufficient food to maintain body weight at 85 percent of their normal weight. None of the rats was zinc deficient during the behavioral testing procedure, Halas says.

Historical examinations by University of North Dakota anatomists Curtis D. Hunt and Jerrolynn C. Kawamoto showed that the hippocampi of rats that were undernourished during fetal development and suckling periods were less developed than hippocampi from the well-nourished rats. Halas says further studies now underway may show

Research psychologist Edward S. Halas uses a 17-arm maze to test zinc-deficient rat for short-term memory. (0484X306-36A)

whether the learning impairments resulting from malnutrition become worse in succeeding generations.

Edward S. Halas is located at the Grand Forks Human Nutrition Research Center, P.O. Box 7166, University of North Dakota, University Station, Grand Forks, N. Dak. 58201—**Ben Hardin,** Peoria, Ill. ■

Mycorrhizae to the Rescue

Potted plants in a greenhouse on the Oregon State University campus at Corvallis are getting help from the VA before they have to face the outside world. Citrus seedlings in an ARS nursery in Orlando, Fla., are making faster progress with the VA. And the VA are enabling wheat in a University of Nebraska greenhouse at Lincoln to better withstand stress and be more productive.

To a growing number of plant scientists, VA does not stand for Veterans Administration. It means vesicular-arbuscular—a type of mycorrhizal fungi. About 400 U.S. and foreign scientists are expected to attend the Sixth North American Conference on Mycorrhizae in Bend, Oreg., June 25-29.

Scientists have known for some time that VA mycorrhizae, or VAM for short, occur naturally on the roots of a wide range of herbaceous and woody plants and actually help the host plant's growth (see *What Are Mycorrhizae?*, next page).

VAM have filament-type structures called mycelia that penetrate plant roots and function as an extension of the root system, taking up moisture and nutrients from the soil for plant use. "VAM mycelia, with their fine structure, can get into places where the root cannot and make available to

Above: Microphotography reveals VA mycorrhizal fungi (VAM) colonizing cortical cells of a plant root. To photograph the root section, root cell pigment was removed and the VAM stained to make them visible. (PN-7094)

Right. To better understand how soil factors influence plant growth and development, researchers at Corvallis, Ore., are conducting experiments in which they split the root system of single plants into two pots. Each pot can be treated separately with any soil factors, including VAM. The plant's root system thus becomes a living bridge to study how these factors interact in the roots. (0484X408-10A)

the plant a lot more water and other nutrients, especially phosphorus," explains microbiologist James R. Ellis at Lincoln. The VAM, in turn, receive nourishment in this symbiotic relationship. In fact, they cannot grow without the plant.

"VAM are a natural part of plant growth and production," says Stanley Nemec, plant pathologist at Orlando. "Almost all plants have mycorrhizae whether they need them or not," he says, noting that every citrus grove tree he has sampled is naturally infected with the fungi. They enable citrus groves to thrive in low-phosphorus soils which are quite common in Florida.

ARS and state agricultural scientists around the country are taking a close look at both sides of this relationship in order to "more fully exploit the benefits in the field and the nursery," says plant pathologist Robert G. Linderman, who heads the multidisciplinary horticultural research program at Corvallis.

Giving Nursery Transplants a Head Start

Led by Linderman, ARS and Oregon State researchers are studying mycorrhizal fungi in combination with various beneficial bacteria to assemble biological aid packages that could be used to produce the best possible plants while reducing dependency on fertilizers.

Work is now focusing on container-produced plants such as geraniums because their environment can be controlled in the greenhouse. Also, many horticultural crops—including nursery stock, bedding plants, and vegetable transplants—are grown in containers. The high per acre value of container-grown plants compared with field-grown plants makes biological aid packages cost-beneficial.

However, it is common in container production to use soilless growth media heavily fertilized with phosphorus. Although VAM naturally increase plant uptake of phosphorus from soil, too much of the mineral inhibits the establishment of and the host response to the fungi.

The Corvallis group sought to determine if VAM could weather these adverse conditions in the pot and still increase a plant's chances of survival and regrowth after it is transplanted into soil where fertilizer levels are lower. Sure enough, they did.

Potted geraniums inoculated with VAM outperformed noninoculated plants grown at the same nutrient levels. By flowering time, the inoculated plants had greater leaf areas

Plant physiologist John R. Potter at Corvallis records readings as biological lab technician Juanita M. Rasmussen uses a photosynthesis chamber to measure the carbon dioxide fixation rate of plants inoculated with VAM. Such measurements help researchers understand how VAM alleviate drought stress. (0484X410-25A)

What Are Mycorrhizae?

Mycorrhizae are fungi that live in the roots of most plants. The relationship between mycorrhizal fungi and their host plant is beneficial to both—perhaps even essential, some researchers speculate, to the host plant's survival and well-being.

In general, mycorrhizae are of two types: endomycorrhizae, which penetrate root cells, and ectomycorrhizae, which live between root cells. Endomycorrhizae, composed largely of the vesicular-arbuscular type (VAM), occur naturally on a wide range of herbaceous and woody plant species. They cannot be grown in artificial cultures and must be produced on living plant roots. Ectomycorrhizae can be grown in artificial cultures, much like mushrooms, but occur on a much more limited host range, primarily trees such as pine, oak, and birch.

The two types are quite different in their biology and response to environmental factors, but both feature an extensive network, or mycelia, of microscopic strands that extend into the soil away from the host plant. These strands, called hyphae, function as an extended root system for the host plant, greatly increasing the plant's ability to gather water and nutrients. They increase the plant's uptake of phosphorus, zinc, and other minerals, reduce the incidence of soilborne diseases and nematode attacks, and increase drought tolerance.

Scientists have already made a good deal of progress in understanding how and when to add endomycorrhizae to container pots and what must be done to ensure their survival and maximize their chances of enhancing host plant survival and growth. Progress has also been made in determining the status of ectomycorrhizae in forests and the effects of their spores on tree growth and development. A recent finding showed that ectomycorrhizae in some way alleviate seedlings from drought stress, possibly by helping the plant to acquire or conserve water.

However, many mysteries about how mycorrhizae operate remain unsolved. Successful use of these biological growth boosters could significantly reduce the amount of fertilizer needed to obtain good, healthy plants, though ironically, it is overfertilization that has suppressed mycorrhizae in the fields from doing what comes naturally.—**Lynn Yarris,** Oakland, Calif. ■

These geraniums are growing in sand fertilized with complete mineral nutrients except for phosphorus. The plants on top, however, were inoculated with VAM and exhibit enhanced growth response under phosphorus limiting conditions. (PN-7095)

and weights, higher root and shoot weights, and more growth uniformity than noninoculated plants. Lower uptake of manganese also resulted from pretransplant inoculation with *Glomus fasciculatum*.This could be useful in reducing manganese toxicity, a situation rare in the field but not unfamiliar in heat-treated nursery soils.

Selecting seed-propagated geraniums as the host plant, Brenda Biermann, then an OSU graduate research assistant, and Linderman tested for four things: whether VAM spread to the roots that grow after transplanting; how long a period is required after inoculation before VAM spores colonize host roots well enough to persist after transplanting; how growth of inoculated transplants is affected by the use of standard container growth media; and whether pretransplant inoculation benefits host growth more than posttransplant inoculation.

To inoculate the geraniums, the researchers mixed either colonized pieces of root or VAM spores into the seedpot media before seeding. They then transplanted the seedlings into pots containing soilless media to simulate nursery production or into pots containing soil to simulate garden planting. Phosphorus was added to the transplants at rates ranging from optimum for nursery production to one-fourth optimum—a level comparable to garden soils.

Even in the soil-transplanted geraniums having one-fourth the optimum phosphorus rate, Biermann and Linderman found that new roots were more rapidly colonized by VAM from the colonized roots than by VAM already present in the soil. A single millimeter of colonized root was sufficient to spread and colonize the host's entire new root system after transplanting. Moreover, the pretransplant colonization took only 2 weeks.

Pretransplant inoculation minimizes transplant shock and reduces mortality, says Linderman. "The transplants take off faster and grow into a salable plant sooner." Inoculation at the time of seeding elicited greater growth response from host plants than posttransplant soil inoculation, he says, even though final levels of root colonization were similar for both pre- and posttransplant inoculations.

The next step, says Linderman, will be to determine if inoculation can induce uniform growth in vegetatively propagated plants of the same clone as it did for seed-propagated plants, which was somewhat of a surprise to the researchers. The results will indicate whether the effect was due to genetic variations in the geraniums or to the VAM.

Diagonally across the United States from Corvallis, in Orlando, Fla., Stanley Nemec is studying the physiology of VAM interactions with citrus seedlings and plans to see if VAM confer any cold-hardiness along with the many other advantages they provide citrus trees. On the practical side, he is working to develop VAM inoculation techniques with several *Glomus* species for commercial citrus tree production.

Plant pathologist Stanley Nemec of the U.S. Horticultural Laboratory, Orlando, Fla., with greenhouse-grown sorghum used to multiply VAM in soil. Recovered VAM are then used to study their interactions with citrus seedlings. (0484X284-4A)

Most citrus trees are started in an outdoor seedbed then transplanted to a nursery row where the scion is budded and grown to salable size. But greenhouse production is rapidly gaining on outdoor production, Nemec says. Trees are seeded and budded in the greenhouse, then either moved to the outdoor nursery row or held indoors and sold to the grower.

Direct inoculation of the seedbed has not been very practical or successful, Nemec says. The seedbed requires large quantities of inoculum that then has to overcome the vagaries of weather (even in Florida) and competition from other fungi and soilborne organisms before it takes hold.

However, Nemec is encouraged with a fluid drilling technique he believes "has a lot of merit." Both seed and VAM spores are suspended in a water-absorbing hydrogel and drilled into the seedbed. The hydrogels, which range from several types of synthetic materials to clay, maintain a moist environment around seed and fungi and keep them packaged together while the seed takes root. At present, hydrogels are primarily used in experimental work and have had only limited commercial use, he says.

Another inoculation technique that is showing a lot of promise after only 2 years of testing is a root dip. When seedlings are removed from the seedbed for transplant to the nursery row, their roots get dipped in a slurry of gel and VAM. This way, says Nemec, the inoculum surrounds the root tips and offers the potential for multiple infec-

tions. And because citrus rootstocks vary in their dependency on VAM, the technique gives a grower the advantage of inoculating only those rootstocks that are VAM dependent.

For greenhouse production, Nemec has had almost complete success in improving the growth of seedlings when he first inoculates the growth media with VAM. However, unlike the Corvallis group, he uses sand or potting mixes with a low level of phosphorus. In 10 out of 13 trials in which he transplanted the seedlings to the outside nursery row, the inoculated seedlings outperformed noninoculated seedlings, he says. He is cooperating with a producer of commercial potting mixes to develop low-phosphorus types.

"Plants inoculated in the greenhouse and transplanted to the field are showing some very exciting results," Nemec says. By fumigating the soil before transplant, he has budded whips that are more then twice as tall as the noninoculated seedlings. When transplanted into nonfumigated soil, the inoculated seedlings still achieve a height one and one-half times taller than noninoculated seedlings.

Nemec plans to try cover-cropping the seedbed to find if another mycorrhizal host will increase VAM populations for the citrus seedlings. He won't have to go far for the host. Almost any plant suited to local growing conditions would do, he says, because almost all plants are infected with VAM.

Helping Wheat Resist Drought

In Lincoln, Nebr., scientists are finding that VAM that live symbiotically on wheat roots play a role in helping wheat crops survive periods of drought.

The challenge now is to determine what is required to transfer this benefit to wheat grown under field conditions.

ARS microbiologist James R. Ellis and colleagues at Lincoln conducted greenhouse experiments that showed VAM enabled wheat to undergo more frequent and longer periods of drought stress and produce yields twice those of uninfected plants. Yield, however, was also affected by the stage of plant maturity during drought stress and subsequent moisture conditions. "This beneficial relationship has also been noted between VAM fungi and sorghum and VAM and grass," says Ellis.

With few cell walls, the mycelia provide a path for rapid movement of water and nutrients to help plants recover from drought stress quickly. And they may have hormonal effects, Ellis says. Some hormones may stimulate root growth. Others may reduce loss of water from the plant by producing hormones that help the plant govern the opening and closing of leaf pores, or stomata.

The scientists are studying relationships among mycorrhizae infections, root development, water and nutrients in the soil, and nutrient uptake in the plant. Nutrients in the studies include nitrogen, phosphorus, calcium, copper, iron, manganese, potassium, zinc, chlorine, and magnesium.

They conducted their greenhouse study in sterilized soil 4 feet deep so they could avoid growth conditions where roots become restricted, and could study certain strains of VAM without competition from others. "Hopefully, this sort of experiment will help us go from greenhouse studies to field studies without a lot of loss of information," says Ellis.

The researchers found that severe reduction in yield of water-stressed wheat was prevented by both *Glomus deserticolum* and *G. fasciculatum*, two common VAM fungi.

"We've also found that some mycorrhizae are a little lazy while others are more active," says Ellis. "They have different abilities and that's why we're looking for a superfungus. If we find one, however, we would need to learn what cultural practices might make it compete well with other VAM in nature."

Related studies at Kansas State University by microbiologist Barbara A. Daniels indicate that crop rotations affect VAM survival rates. Although wheat benefits from VAM, she found that infection is low when wheat is planted continuously.

In tillage studies Ellis has found VAM populations in the top 6 inches of soil slightly greater on land that is in no-till fallow and treated with herbicide to control weeds than in plowed or subtilled fallow soil. In sod he observed even higher populations in the top 6 inches, but below that populations dropped lower than in any tillage system.

In a greenhouse at the University of Nebraska, Lincoln, microbiologist James R. Ellis (right) and research associate Harold Larson study wheat inoculated with VAM. Sections of ordinary irrigation pipe serve as soil plots to allow unconfined root growth. (0384X171-22A)

The extent of VAM infections may also be governed by the plants' need for them. Ellis said that apparently an interaction between VAM and plants controls infections in a way that keeps the biological systems from wasting energy.

In an area where soil fertility is low, VAM help plants overcome mineral deficiencies. Is there any danger that VAM could deplete soils of trace minerals? "I don't think so," Ellis replies. "Soil fertility specialists generally seem less sold on replacing trace minerals than they once were. We have a big reservoir of trace minerals in our soils. The problem is making the minerals that are present available to the plant."

James R. Ellis is located in Room 119, Keim Hall, University of Nebraska, East Campus, Lincoln, Nebr. 68583. Stanley Nemec is at the Horticultural Research Laboratory, 2120 Camden Road, Orlando, Fla. 32803. Robert G. Linderman is leader of the Horticultural Crops Research Laboratory, 3420 S.W. Orchard Ave. Corvallis, Oreg. 97330—**Lynn Yarris**, Oakland, Calif., **Ben Hardin**, Peoria, Ill., and **Judy McBride**, Beltsville, Md. ■

Lasers Could Shed Light on Crop Deficiencies

Lasers on satellites won't replace the plow or cow, but they could some day help farmers reap better harvests. That's one possible outcome of a unique project using lasers to detect nutrient deficiency in crops.

Plant material (and other substances) exposed to certain wavelengths of light or other forms of energy will sometimes emit its own light. Electrons orbiting the nuclei of atoms in plants absorb some of the light energy, become "excited," and "jump" to a higher orbit around the nuclei. Often, these new orbits are unstable: the electrons quickly fall back to their original state and emit the extra energy as light of a specific, usually new, wavelength. This is the principle behind fluorescent lighting.

Cooperative research among scientists at the Beltsville Agricultural Research Center, the nearby Goddard Space Flight Center, and a private consultant in Landover, Md., demonstrates the ability of using laser light to detect nutrient imbalances through changes in the leaves' fluorescence. The fluorescence occurred primarily in the chlorophyll pigments.

Agronomist James E. McMurtrey III of Beltsville's Field Crops Laboratory says the team detected corn suffering from a lack of nitrogen, iron, potassium, or phosphorus, and soybeans suffering from a lack of phosphorus, boron, sulfur, calcium, magnesium, or potassium. In the laboratory tests, they used a pulsed nitrogen-gas laser emitting a beam of ultraviolet light on greenhouse-grown plants.

"Some of the deficient plants have a unique fluorescent response that could possibly allow us to identify the missing nutrient from miles above the ground," McMurtrey explains.

McMurtrey, NASA scientists E.W. Chappelle and F.M. Wood, and private consultant W.W. Newcomb used ultraviolet laser light with a wavelength of 337 nanometers (a nanometer, "nm," is one-billionth of a meter, or about 4 hundred-millionths of an inch). Testing 7-week-old normal corn and soybean plants, the scientists found that the

ARS agronomist James E. McMurtrey trains a nitrogen laser beam onto soybean plant leaves. Cooperating physical science technician Frank Wood from NASA operates the telescopic detection system which reads flourescence from the leaves. Nutrient-deficient leaves show a significant difference in fluorescence. (0284W117-8)

Nutrient Deficiency	Fluorescence					
	Increase	Decrease	Inc.	Dec.	Inc.	Dec.
Nitrogen		C		C		C
Iron		C		C		C
Potassium	S	C	C S		C S	
Phosphorous		S		C S		C
Boron				S		
Sulfur				S		
Calcium			S		S	
Magnesium			S		S	
	440nm		690nm		740nm	

C Corn S Soybeans

PN-7096

greatest amount of light given off by the crop plants had wavelengths of 440, 690, and 740 nm.

They then trained the laser onto the leaves of nutrient-deficient plants. Each of seven groups of plants was deficient in a particular nutrient. When compared to fertilized plants at the

three wavelengths, corn showed significant decreases or increases in fluorescence for four nutrients, and soybeans showed these differences for six nutrients. (See graph.) McMurtrey says he is most confident of the results for nitrogen, phosphorus, and especially potassium, because its absence dramatically increased fluorescence at two of the wavelengths.

Though the mechanisms governing these fluctuations in laser-induced fluorescence are not well understood, McMurtrey and Chappelle foresee practical use of what is already known. Crops growing in soils suspected of being deficient in certain nutrients could be surveyed with lasers from high-altitude aircraft or satellites. They also hope to be able to detect the level of deficiency so that the amount of fertilizer could be recommended.

Work undertaken by the USDA, NASA, and other organizations has paved the way for the remote sensing that is currently being used to monitor our agricultural resources. Laser systems of sensing could greatly enhance the methods currently in use, they say. However, the cost of developing these systems would be greatly decreased if multiple uses could be found for defense, agriculture, and other users.

In the future, the team will take their research to the field, and they will attempt to apply laser-induced fluorescence to other crops and crop stresses such as disease, insect damage, or drought. Aside from the practical benefits gained by spotting crop problems, the scientists hope their research will bring them closer to unraveling the biochemical riddle of photosynthesis. Future research should help explain the effects of nutrients (or their absence) on the several forms of chlorophyll and other, still-unidentified molecules that play key roles in this dynamic process.

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Model Predicts Annual Stripe Rust Disease Level

For 5 straight years now, researchers have accurately predicted the severity of stripe rust in winter wheat throughout six western states with a statistical model.

The stripe rust model can be used with spring wheat as well as winter wheat and should have global applicability, say its developers. Concepts behind the model could also be applicable to diseases in other crops.

Developed by ARS plant pathologist Roland F. Line of the Wheat Breeding and Production Research Laboratory at Pullman, Wash., and Stella M. Coakley, a plant pathologist supported by the National Science Foundation at the National Center for Atmospheric Research, Boulder, Colo., the model is based on a correlation between winter and spring temperatures in the West and the incidence of stripe rust in this region. The researchers are working on linking the model with Agnet, the national agricultural computer network.

Stripe rust, a highly explosive fungal disease of grains, ranks as one of the world's most serious wheat diseases. Because of current management practices, stripe rust is now present in wheat fields all year round, ready to strike under the right climatic conditions, says Line. Growers provide a perpetual host with winter-spring wheat rotations, early and late planting, and summer planting for erosion control and livestock feed. In addition, volunteer wheat harbors the fungus.

Line and Coakley established a quantitative relationship between climatic variables and stripe rust. They did this by monitoring the intensity of rust occurrences in fields located mainly in eastern Washington and Oregon, where stripe rust is most prevalent, but also at sites in Idaho, Montana, Colorado, and California. These data were compared and correlated to monthly and seasonal temperature and precipitation data, including snowfall and depth of snow on the ground.

Temperature, they found, was the key correlating factor. Says Line, "Higher than normal temperatures in the winter coupled with lower than normal temperatures in the spring spell severe rust problems." This combination can reduce the effectiveness of even adult rust resistance in a wheat variety. Several years ago, Line and his coworkers discovered this high-temperature-dependent type of rust resistance in mature plants that does not succumb to new races of rust.

Wheat leaf heavily infected with stripe rust pustules. (0983X1278-24)

Rainfall, he says, was not a factor in either the frequency or the amount of stripe rust occurrence in their study.

To determine the cumulative effect of temperature on stripe rust development, Coakley and Line used a measurement called "degree days." Stripe rust germinates best and infects wheat plants most readily at 44°F (7°C). For their model, the researchers subtract this figure from the average daily temperature to get either negative (when the result is zero or less) or positive (when the result is greater than zero) degree days. They then sum the negative or positive degree days, starting in September and continuing throughout the growing season of the particular wheat variety being monitored.

AgResearch Notes

Plump wheat kernels at left are from a nonsusceptible line. Shriveled kernels at right are from a susceptible line. Both wheat lines were grown at the Washington State University Spillman Agronomy Research Farm. (0983X1279-21A)

From their studies, the researchers found that the accumulated negative degree days in the months of December and January and positive degree days during the 80 days following the onset of spring determine how widespread and how severe a stripe rust epidemic will be by harvest. The onset of spring is determined by degree days rather than by the calendar, Line explains, because it will vary depending on the region. The first 2-week period that accumulates 40 positive degree days is considered the first 2 weeks of spring.

Line and Coakley put these findings to use in a statistical model that enables them to make preliminary predictions for the winter wheat disease level as early as January.

"The use of this model enables guidelines to be offered on how each variety of wheat is likely to be affected by stripe rust, which can help growers decide whether or not to treat their fields, and if so, when would be the best time to do so," says Line.

"This information, plus the 1982 registration of a fungicide for rust control and the use of resistant varieties, may be able to reduce much of the damage to wheat caused by stripe rust," he says. The recently registered fungicide is the result of other research by Line.

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Longstanding Range Grasses

By tracking down the location of long-abandoned research plots, a team of federal and state scientists has provided range managers with specific recommendations of grass best suited for long-term survival.

Most of the seeding plots examined date back to the late 1940's and early 1950's. "Establishing a reliable picture of grass persistence usually requires at least 10 years' observation," says ARS range scientist William J. McGinnies, who headed the team. "At any location, certain grasses will become established and do relatively well in periods of above-average precipitation. During any 10-year period, however, 1 or more years of below-average precipitation usually occur and we can see which species have the ability to withstand drought."

McGinnies, along with Wendell G. Hassell, USDA Soil Conservation Service, Denver, Colo., and Clinton H. Wasser, Colorado State University, Fort Collins, examined 60 seeding tests that were conducted at 45 locations throughout Colorado. They rated more than 200 grass species planted in 15 different vegetative zones. As a result, some earlier recommendations have been revised because, after many years, other species proved to be hardier and more productive.

The findings are published in CSU Special Series 21, entitled "A Summary of Range Seeding Trials in Colorado." Included with the stand or persistence rating, is the plot location, specific native vegetation zone, soil type and land slope, elevation, average annual precipitation, vegetative and cropping history, date planted, planting procedure, and a brief history of the plots.

Plot summaries have been categorized on the basis of vegetation zones. The 15 zones are described in the text and outlined on a map. Where range site (a land classification system used by the SCS and other agencies) is known, a cross-index shows all study plots located in that range site. Thus, once the vegetation zone or range site is determined, all of the applicable test results can be quickly located.

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Alaskan Growers Can Nip Weed Problems in the Bud

Farmers in Alaska now clearing their land for cultivation have a golden opportunity to minimize future weed problems. An ARS survey has shown that the comparative youth of cultivated fields in our 49th state gives farmers there a chance to take control of their weed problems before those problems really begin.

Most of us in the contiguous states tend to think of Alaska as a sprawling iceburg, populated primarily by wolves, bears, seals, and moose, with a few oil pipelines latticing its wasteland. Actually, it has been estimated that there are at least 20 million acres of land in Alaska suitable for farming, and the state has set a goal of bringing over half a million acres of this land into production by 1990.

In a survey of 84 agricultural fields representing a wide number of crops, ARS agronomist Jeffery S. Conn, along with University of Alaska agricultural assistant John DeLapp, both at Fairbanks, found that native weeds dominate recently cleared fields but yield to introduced weed species the longer the fields remain in cultivation.

Spring cultivation, the researchers say, can control native weed species, and the use of weed-free crop seed and livestock feed (which can spread weed seed through manure) can keep introduced species out.

Says Conn, "The results of the survey allow us to better target weed control and biology research by identifying the most pressing weed problems. For example, we found that total weed cover is low in the first few years of cultivation but increases as introduced weed species become more prevalent."

In addition to spring cultivation and the use of weed-free seed and feed, Conn says farmers can also "sanitize farm machinery coming from weed-infested areas to reduce interfield transfer and cut back on opportunities for colonization by introduced weed species."

All of these practices together, he says, "should result in higher quality crops, less competition from weeds, and less expense for future weed-control measures."

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Erosion Control Plus Profits in Pacific Northwest

Wheat growers in the intermediate rainfall areas of the Pacific Northwest can control erosion and increase profits by switching from winter to spring grains.

Most growers currently practice a winter wheat-fallow rotation in this region. Though highly productive, it has led to severe erosion that, if left unchecked, threatens to one day exhaust the fertile, irreplaceable topsoil crops need.

ARS agronomist Allen J. Ciha, Pullman, Wash., is studying cropping systems that can help control erosion. He found that switching to yearly spring grain production offers several advantages over the current system, provided there is enough annual precipitation.

"For areas receiving about 13 to 18 inches of rainfall annually," says Ciha, "spring wheat yields are high enough to more than meet the extra seeding and harvesting costs of an annual cropping system."

The greatest advantage of switching to spring grains is economic: a crop is obtained each year instead of once every other year. With sufficient rainfall, the added yield of the extra crop exceeds the higher per year yield of winter wheats. For example, a good winter wheat can be expected to yield 50 bushels per acre, while the best spring wheats might yield only 30 to 35 bushels per acre. However, with the addition of the second crop, growers can get 60 to 70 bushels per acre of spring wheat during a period of time they would be obtaining 50 bushels per acre of winter wheat.

In areas with 12 inches or less annual precipitation, Ciha cautions that spring yields would be only about 15 to 20 bushels per acre, which, with the additional seeding and harvesting costs plus other management expenses, would not be profitable.

Other benefits of switching to a spring wheat cropping system include control of winter annual grassy weeds, fewer disease problems associated with early seeding of winter wheat, and no winter injury problems.

Cropping to spring wheats to control erosion works best when minimum tillage—generally, a chisel tillage operation—is used in the fall. "This will break up the soil enough to allow better absorption of rainwater while leaving plenty of residue [straw] on the surface to hold water and reduce runoff," says Ciha.

Performances of commerical spring wheat varieties when used in this cropping system vary, but Ciha says that any of the area's leading varieties will work fine. Already some growers in the area are trying the system.

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Dairy Cows Culled Too Soon

Contrary to the "too little too late" syndrome troubling so many industries in this country today, the dairy industry may be suffering from too many too soon: too many cows being culled before producers realize a full return from their breeding programs.

ARS animal scientist Robert C. Lamb, Logan, Utah, has been running computer studies of cattle age distribution in U.S. dairy herds and its relationship to milk production. "We're learning," he says, "that the management programs designed for optimum genetic progress are not necessarily the best programs for optimum economic progress."

"A dairy cow does not reach her peak milk production until the age of 6, but most animals in this country are removed from the herd after their third lactation—about 4 years of age. At this age, they are reaching only about 90

044-40-15

percent of their production potential."

The idea, according to Lamb, has been to replace, as rapidly as possible, animals from one generation with animals from the succeeding generation in order to take advantage of the new generation's genetic superiority. "Genetic progress is a fact," says Lamb. "Though there will be individual exceptions, for the overall herd, each new generation should be better producers than their predecessors. However, this genetic production gain from young stock might not exceed the production of a mature cow."

Lamb recommends that dairy farmers cull cows that are poor producers or breeders, but keep profitable animals longer. Once a cow reaches her peak, decline is slow and her production should still be high at 8 years of age, he says, noting that European producers annually cull only about 15 to 20 percent of their cows, compared to a 30- to 40-percent culling rate practiced in this country. And they operate at a profit.

Lamb plans to continue this study. Determining the best age distribution of animals in a herd is necessary for a healthy balance between genetic and economic progress which, in turn, is necessary for a healthy U.S. dairy industry.

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The female omnivorous looper, unlike her close relatives, carries her sex gland on the outside of her body. When ARS chemists Leslie M. McDonough initiated a project to identify the sex pheromone of this avocado-loving insect pest, the first step was to dissect its pheromone-secreting gland. That posed a problem: no one knew for certain where it was.

ARS physical science technician Connie L. Smithhisler, Yakima, Wash., working with entomologists Blair Bailey and Mike Hoffmann of the University of California, Riverside, located the gland by a combination of tissue studies and electroantennagrams. In the latter, the researcher attaches tiny electrical leads to a male looper's antenna to determine if gland extracts cause a response. A strong response confirms the presence of the pheromone.

Smithhisler found that the looper's gland is actually similar to the glands of other moths—"a ring of cells located in the intersegmental membrane between the eighth and ninth abdominal segments."

Once located, McDonough went on to determine the pheromone's chemical structure and synthesize an identical copy. The synthetic pheromone has been successfully field-tested and can now be used to monitor looper populations or thwart their mating attempts.

A native Californian that has been content to stay there, the omnivorous looper lives up to its name. It enjoys a varied diet encompassing dozens of ornamental and fruit trees and shrubs—with a particular penchant for avocados. In large numbers, loopers can completely defoliate an avocado grove.

Leslie M. McDonough and Connie L. Smithhisler are located at the Yakima Agricultural Research Laboratory, 3706 West Nob Hill Blvd., Yakima, Wash. 98902.—**Lynn Yarris,** Oakland, Calif. ■